NEUROSECRETION IN THE THORACIC GANGLION OF THE CRAB, ERIOCHEIR JAPONICUS

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In a study of sacculinization in *Charybdis japonica* (Matsumoto, 1952) the author reported that the thoracic ganglia of the host crabs are damaged by the root system of the parasites, *Heterosaccus papillosus*. The same kind of damage is also observed in other species of sacculinized crabs. More recently, in a study of epicaridization in the fresh water crab, *Eriocheir japonicus* (Matsumoto, 1953), it was observed that the thoracic ganglia of male hosts become greatly deformed by the pressure of the parasites, *Entionella fluviatilis*. In order to elucidate the nature of the abnormalities found in the ganglia of crabs subjected to so-called parasitic castration, a detailed histological study of the thoracic ganglion of normal animals became necessary. In the course of this work the presence of neurosecretory cells was observed in the thoracic ganglion of *Eriocheir japonicus*. While Enami (1951b) found only one type of neurosecretory cells (α cells) in the thoracic ganglion of the crab *Sesarma*, that of *Eriocheir* seems to contain three cytologically different types of these cells. Their description, in the present paper, appears of interest, since differences in cytological appearance may indicate different functions of the cellular products. Furthermore, these cells exhibit signs of a mode of discharge of the neurosecretory material which has so far been observed only in vertebrates.

Materials and Methods

Eriocheir japonicus is a grapsoid crab, commonly found in the fresh waters of Japan; animals collected at the Asahi River in Okayama City were used for this study. The observations are based on sectioned tissue of thoracic ganglia. The crabs used (80 males and 72 females) were in various stages of development. They ranged from 5 to 45 mm. carapace length. The thoracic ganglia were fixed in Bouin's solution or Zenker-formol and cut into serial sections of 8 μ thickness by the usual paraffin method. They were stained with Gomori's chrome-alum hematoxylin and phloxine or Delafield's hematoxylin and eosin. Besides these methods some materials were fixed in Susa or trichloracetic acid and were stained with Mallory's triple stain or Masson's trichrome stain in order to compare them with those described in Enami's (1951b) study.

Observations

1. *Types of nerve cells and their location in the thoracic ganglion*

There are four kinds of nerve cells in the thoracic ganglion of Eriocheir each of which shows a definite localization. For the time being these cells are designated as types A, B, C, and D. Their distribution is shown diagrammatically in Figure 1. A-type nerve cells are giant elements with diameters of 80–100 μ in the adult and are mainly found in the medial and posterior parts of the ganglion. B-type nerve cells are small with diameters of 15–20 μ and are distributed all over the ganglion. They are mingled with the A-cells in the medial and posterior parts. In the anterior part, many B-cells are found on the ventral side. C-type nerve cells are also small with diameters of 10–20 μ; they are located in paired groups at the anterior end of the dorsal side of the ganglion. D-type nerve cells are minute and are arranged in densely packed, paired masses ventral of the C-cells.

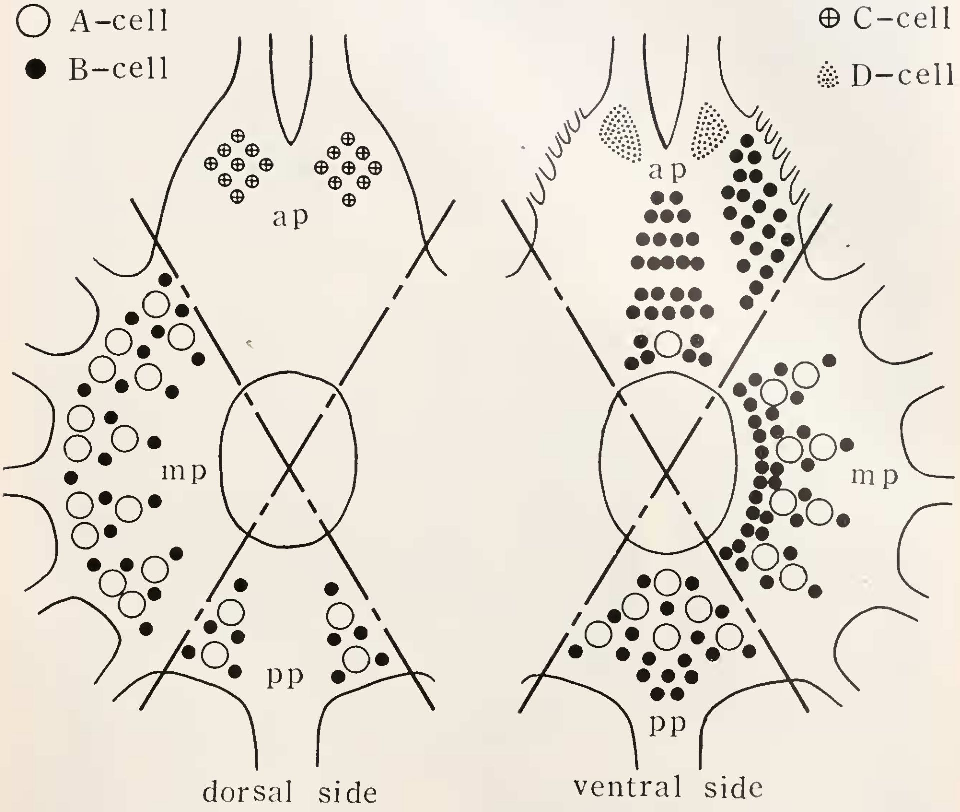
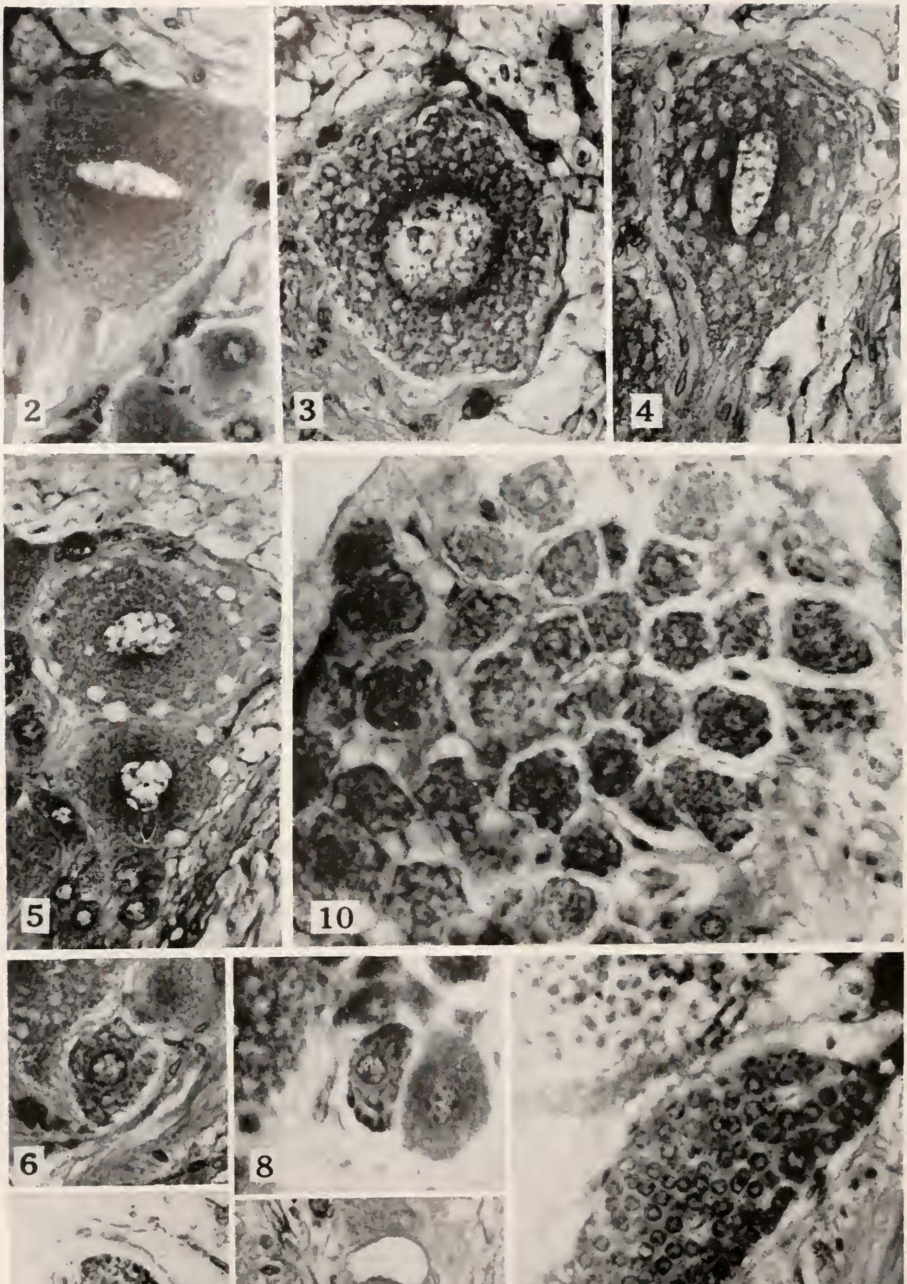


FIGURE 1. Diagrammatic illustration of the distribution of four types of nerve cells in the thoracic ganglion of *Eriocheir japonicus*. The dorsal and ventral sides of the ganglion are shown separately. Each diagram is subdivided into three parts, anterior (ap), median (mp), and posterior (pp), for convenience in description.

2. *Neurosecretory activity of different cell types*

A-cell: The giant A-cells are considered to be neurosecretory on account of cytological features such as modifications of their nuclei, the appearance of minute granules, and the occurrence of small vacuoles. Some giant cells in the thoracic ganglion contain nuclei which differ considerably from the round nuclei of ordinary cells. These modified nuclei may be flat or crescent-shaped; as a rule, their contents



Figures 2–11.

show no affinity for nuclear stains. Many minute dark blue granules are seen to gather closely around the crescent-shaped or flat nuclei in preparations stained with Gomori's method (Fig. 2). In the cytoplasm, the occurrence of small vacuoles is noticeable. Some giant cells have a coarse cytoplasm as shown in Figure 3. This appearance is considered as typical of a stage preceding that marked by vacuolated cytoplasm illustrated in Figure 4. The latter seems to be the most vigorous stage of secretion. Many small vacuoles appear over the whole cytoplasm; some vacuoles stain pale violet, others seem quite empty. This figure resembles one depicting certain neurosecretory cells in the suboesophageal ganglion of the cockroach, Leucophaea, studied by B. Scharrer (1941a, Fig. 2C). Certain other A-cells have only small vacuoles in the periphery of the cell body but never in the central portions (Fig. 5).

Judging from all these figures, it may be assumed that the minute granules which appear in the vicinity of the modified nuclei spread over the entire cytoplasm, while simultaneously many small vacuoles containing neurosecretory substance appear in the cytoplasm; then the vacuoles gradually migrate toward the periphery of the cell and disappear. This seems to be the mode of discharge of the neurosecretory material in these cells; it will be discussed in later sections of this paper.

During these secretory cycles apparent in the cytoplasm, the nucleus shows concomitant changes of its shape, *i.e.,* the round nucleus gradually becomes flat or crescent-shaped during phases of cytoplasmic activity and then expands and rapidly regains its round shape during the resting stage of the cytoplasm. The nucleus may, therefore, be considered as playing an important part in the neurosecretory activity of the A-cells. These various pictures are commonly found in the thoracic ganglion of the normal adult crab.

B-cell: B-cells are not only much smaller than A-cells but they also show a different kind of neurosecretory behavior. The secretory activity of B-cells is illustrated in Figures 6–9. Since all photomicrographs shown in this paper have the same magnification, differences in cellular size can be readily appreciated.

FIGURE 2. Giant neurosecretory cell (A-cell) showing many minute dark granules surrounding a flat nucleus. Male, 30 mm. carapace length. Bouin, paraffin, 8 μ, Gomori's chrome alum hematoxylin phloxine. Photomicrograph, × 410. Figures 3–9 are from the same specimen, shown at the same magnification.

FIGURE 3. A-cell with coarse cytoplasm. The minute granules are scattered throughout the cytoplasm and the nucleus has regained its round shape.

FIGURE 4. A-cell with many small vacuoles in the cytoplasm. The ellipsoid nucleus is typical of this stage of secretory activity which is interpreted as the most vigorous in the cycle.

FIGURE 5. A-cell showing vacuoles only in the periphery of the cell. This figure may represent the last stage in the secretory cycle.

FIGURE 6. Small neurosecretory B-cell with granulated cytoplasm containing many irregular masses.

FIGURE 7. B-cell containing dark staining larger masses in the cytoplasm.

FIGURE 8. B-cell in which two dark staining large masses are concentrated in the cell periphery.

FIGURE 9. B-cell in which the dark cell inclusions have been replaced by two large vacuoles.

FIGURE 10. A group of secreting C-cells. These seem to correspond to Enami's β cells. Male, 31 mm. carapace length. Zenker-formol, paraffin, 8 μ, Gomori's chrome alum hematoxylin phloxine. Photomicrograph, × 410.

FIGURE 11. A mass of minute D-cells which never show any secretory activity. Note round nuclei of uniform size. The cytoplasm is scarce and the cell boundaries are difficult to discern. Same specimen as shown in Figure 10. Photomicrograph, × 410.

The first stage in the secretory cycle of B-cells may be that in which the cytoplasm as a whole becomes granulated and many small irregular masses appear in it, as shown in Figure 6. No changes in the nucleus are observed. In the next stage, these irregular masses show a tendency to aggregate into larger, darker staining masses (Fig. 7). Presumably the third stage is depicted in Figure 8; the granular material is concentrated into one or two large dark masses at the edges of the small cell. Figure 9 illustrates the last stage of secretion. The dark masses have disappeared, and large vacuoles remain in their place. The nucleus does not show any signs of activity during the secretory process. The structure of the cell shown in Figure 9 closely resembles the photomicrograph of a neurosecretory cell in the suboesophageal ganglion of *Blaberus craniifer* published by B. Scharrer (1941a, Fig. 6).

These signs of neurosecretory activity are not observed in all B-cells present in the thoracic ganglion, but there are regional differences. The secretory activity of B-cells seems most pronounced in the posterior part; it is less frequently observed in the anterior part, and rather rare in the median part.

C-cell: C-cells have small granules and droplets in the cytoplasm which stain with aniline blue. The number and size of the granules and droplets vary, but in their general appearance the C-cells resemble Enami's β cells. When fixed in Zenker-formol and stained according to Gomori, the C-cells show a characteristic lumpy cytoplasm with black minute granules and small droplets (Fig. 10). The C-cells are much less frequent than the A- or B-cells, but they all show signs of secretory activity as described above.

D-cell: D-cells are the smallest nerve cells in the thoracic ganglion. The nuclei are round and, for the most part, approximately uniform in size and appearance. The cytoplasm is not abundant but stains deeply with basic dyes; the cell boundaries are difficult to discern. These cells form two small, densely packed masses as illustrated in Figure 11. They show no secretory activity.

3. *Capillary networks in the thoracic ganglion*

It is a matter of common knowledge that the crustaceans possess an open circulatory system. There are several main arteries originating from the heart; these open into the haemocoel, and no capillaries are found in the majority of the organs. Within the thoracic ganglion, however, there are exceedingly well developed capillary networks. They surround individual giant nerve cells, and enclose groups of two or three smaller cells. Figures 12 and 13 show examples of these capillary networks in the thoracic ganglion of Eriocheir. In Figure 12 capillaries are seen to enclose giant nerve cells, in Figure 13 groups of smaller cells. This capillary network branches out from five or six small arteries which enter the thoracic ganglion in the mid-ventral region and pass through it to the dorsal side. The rich capillary bed forms plexus around ventrally and dorsally located neurosecretory cells; it also penetrates the entire medulla of the ganglion. On the dorsal side certain capillaries join small vessels which, on leaving the ganglion, open into the haemocoel; others empty directly into the haemocoel at the periphery of the ganglion.

Such a special arrangement of the circulatory system of the thoracic ganglion suggests special functions. Neurosecretory cells must be expected to have a high metabolism and, therefore, require a rich blood supply. This is also the case in vertebrates where nuclei composed of secreting nerve cells are among the most richly vascularized of the central nervous system (see Scharrer and Scharrer, 1954). Furthermore, it seems that in the crab studied here, neurosecretory substances are given off at the periphery of the cell into these capillaries and thus reach the general circulation of the body.

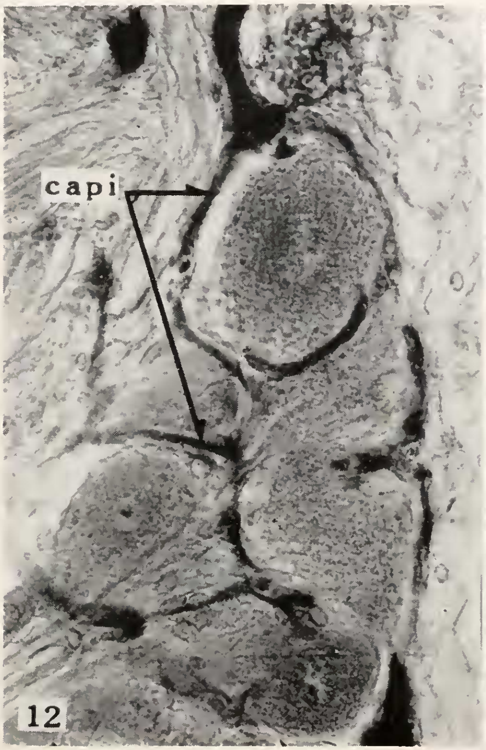


FIGURE 12. Capillaries closely surrounding individual giant neurosecretory cells within the thoracic ganglion of Eriocheir. Female, 25 mm. carapace length. Capi, capillary. Zenker-formol, paraffin, 8 μ, Masson's trichrome stain. Photomicrograph, × 410.

FIGURE 13. Capillaries enclosing groups of two or three small cells in the thoracic ganglion of the same specimen as shown in Figure 12. Capi, capillary. Photomicrograph, × 410.

DISCUSSION

The histological examination of the thoracic ganglion of *Eriocheir japonicus* showed the presence of three types of neurosecretory cells. In a recent publication, Enami (1951b) studied neurosecretion in the central nervous system of Sesarma and reported that only one type of neurosecretory cells (α cells) is found in the thoracic ganglion of this crab. On account of their size, the giant A-cells in the thoracic ganglion of Eriocheir would seem to correspond to the α cells of Sesarma. But the morphological characteristics of these two cell types are quite different;

in particular, the central body of the α cell is never found in A-cells of Eriocheir, even when the same fixation and the same staining methods are used.

The C-cells in the anterior region of the thoracic ganglion of Eriocheir have the same histological structure as Enami's β cells. But in Sesarma, these cells according to Enami (1951b) occur in the optic ganglia, the brain, and the commissural ganglia, but never in the thoracic ganglion. These differences in cellular distribution may be considered as genus differences.

Some cellular details in the A-cells and B-cells of Eriocheir are quite similar to those in the suboesophageal ganglion of cockroaches studied by B. Scharrer (1941a). Thus neurosecretory processes in insects and crustaceans seem to have certain features in common.

Concerning the discharge of neurosecretory substances, two possible ways were considered in earlier studies on neurosecretion. Scharrer and Scharrer (1945) in their review on neurosecretion described as one way the discharge of the secretory substances into the capillaries, and as the other a transport of neurosecretory material along nerve fibers. The latter way, *i.e.,* the movement of neurosecretory substances along axons, was confirmed by many recent investigators.

These observations resulted in the concept of neurosecretory systems, *i.e.*, the hypothalamic-hypophyseal system of vertebrates (Bargmann and Scharrer, 1951), the intercerebralis-cardiacum-allatum system of insects (Scharrer and Scharrer, 1944; B. Scharrer, 1952), and the neurosecretory system of crustaceans (Bliss, 1951; Bliss and Welsh, 1952; Passano, 1951). Because of its physiological implications this concept received considerable attention in recent work on neurosecretion. By comparison, less emphasis was placed on studies demonstrating the direct discharge of neurosecretory substances from the cell surface into surrounding capillaries or tissue spaces. There are many reported cases of neurosecretory activity in which the mode of discharge of the secretory product either was not studied in particular or could not be determined; for example in the neurosecretory cells of the stellate ganglion in vertebrates (Eichner, 1952) or of the central nervous system of Limulus (B. Scharrer, 1941b).

With regard to crustaceans, Enami (1951b) suggested an axonal transport of neurosecretory material, and Bliss and Welsh (1952) came to the conclusion that in decapod crustaceans neurosecretory substances produced in various parts of the central nervous system migrate along nerve fibers to the sinus gland where they are stored and released.

The present study furnishes evidence of a different mode of discharge of neurosecretory products in crustaceans. It is of considerable interest that, although the circulation in crustaceans represents an open system, capillaries were found to surround neurosecretory cells in the thoracic ganglion of Eriocheir. Moreover, the neurosecretory substances produced in the perikaryon gradually seem to move to the periphery of the cell and there to disappear. From these observations it may be concluded that in the thoracic ganglion of Eriocheir, neurosecretory material is given off directly into the surrounding capillaries and does not migrate along nerve fibers. Thus both ways of discharge of neurosecretory products, as first described by Scharrer and Scharrer (1945, 1954) for vertebrates, have now been established also for invertebrates with an open circulatory system.

The physiological significance of the three types of neurosecretory cells in the thoracic ganglion of Eriocheir is as yet unknown. Smith (1948), Enami (1951a), and Brown and his collaborators (Brown, 1949, 1950; Brown, Sandeen and Webb, 1949; Sandeen, 1950) demonstrated the existence of chromatophorotropins in the thoracic ganglia of crustaceans. Furthermore, Brown and Cunningham (1941) reported that neurosecretory cells in the central nervous system of Limulus furnish chromatophorotropic principles. Hence, it seems reasonable to assume that some of the neurosecretory cells in the thoracic ganglion of Eriocheir may be the source of chromatophorotropic principles.

In recent studies of sacculinization and epicaridization of crabs (Matsumoto, 1952, 1953), the thoracic ganglia of the hosts, which had changed to an intersexual condition, were found to be damaged by the presence of the parasites. The deformed ganglia contained fewer nerve cells than normal ones, and their distribution was disarranged. It seems possible, therefore, that a relationship exists, either direct or indirect, between the neurosecretory activity in the thoracic ganglion and the development of secondary sex characters, but this possibility needs further exploration.

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SUMMARY

1. The different types of nerve cells occurring in the thoracic ganglion of the fresh water crab, *Eriocheir japonicus*, their distribution, neurosecretory activity, and the mode of discharge of the neurosecretory substances were studied.

2. There are four types of nerve cells which show a definite localization in the thoracic ganglion; three of them are considered to be neurosecretory cells. Giant A-cells are interpreted as neurosecretory cells on account of the cyclic changes of their nuclei and the gradual movement of many small vacuoles toward the cell periphery. B-cells are small cells showing secretory cycles: numerous granules appear in the cytoplasm, then concentrate into one or two masses at the edge of the cells, and finally disappear leaving large vacuoles in their place. Their nuclei show no changes. C-cells are also small neurosecretory cells; they are thought to correspond to Enami's β cells. The minute D-cells do not posses characteristics to suggest a secretory activity.

3. Several small arteries enter into the thoracic ganglion at the mid-ventral region and pass through to the dorsal side, branching out into many capillaries. These capillaries form networks and closely surround the neurosecretory cells.

4. From these observations it is concluded that the neurosecretory substances in these cells are given off into the capillaries and thus reach the general circulation of the body. This mode of discharge of the cellular product is of interest in view of comparable mechanisms in vertebrates.

5. The physiological activities of these neurosecretory substances in the thoracic ganglion of Eriocheir are as yet unknown.

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